[rom *the* PROCEEDINGS OF THE ROYAL SOCIETY, B, VOL. 82, 1910.]

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Y M. HAALAND, M.D.

[*Reprinted from the* PROCEEDINGS OF THE ROYAL SOCIETY, B. Vol. 82]

The Contrast in the Reactions to the Implantation of Cancer after the Inoculation of Living and Mechanically Disintegrated Cells.

By M. HAALAND, M.D., Imperial Cancer Research Fund.

(Communicated by Prof. J. Rose Bradford, Sec. R.S. Received January 17,—Read February 3, 1910.)

The object of the present paper is to show the difference obtaining between the employment of the living cell, cancerous and normal, as an agent to induce active resistance to the implantation of cancer cells, and the employment for the same purpose of the protein obtained from these cells by mechanically disintegrating them. As a means of devitalising the cells with least disturbance of their chemical properties, the method adopted was that of grinding them either in the MacFadyen-Rowland apparatus at the temperature of liquid air, or in a mortar, cooled by embedding it in ice and salt. Comparison of the results obtained by both methods showed that the latter was the more efficacious. By interrupting the freezing, the material can be kept of a pasty consistence specially favourable for crushing all the cells. The completeness of the disintegration of the cells was ascertained by microscopic examination, revealing the absence of intact nuclei, and, in the case of the cancer tissue, also by inoculation of the material not yielding tumours. Every precaution has been taken to employ mice as uniform in age, size, and weight as possible, in order to make the estimations upon a soil of uniform natural resistance.

In order to analyse the results more closely in each experiment, the animals were killed after the same interval of time, when the tumours threatened to ulcerate, and all the tumours obtained were weighed. The protocol of such an experiment is given, showing all the details of the experiment, including: (1) The length of time elapsed between inoculation and the date when mice were killed; (2) number of tumour mice alive up till then; (3) total number of mice alive; (4) total weight of tumour obtained; and (5) average weight of mice. To obtain an estimate of the average growth, two calculations have been made: the one indicates the average weight of tumour obtained, calculated on all the animals of the series living up till the time when they were killed (negative included): ("average weight of tumours *pro* mouse"); the other gives the average weight of each tumour, when only the positive mice are taken into account ("average weight of tumours *pro* tumour"). It seemed inadvisable to use the figures obtained

for more elaborate calculations, because of the only approximate value of a such figures obtained by biological experiment. In all transferences of cance cells, small factors, such as health of the animals, intercurrent diseases— factors which are incapable of exact measurement—play a very importan part in determining the results.

The accompanying table illustrates the nature of results obtained, after th inoculation of 0·10 c.c. of material, devitalised by grinding for $1\frac{1}{2}$ hours a the temperature of liquid air, when the mice are tested 15, 17, and 20 day later, with varying doses, 0·02 to 0·05 of mammary carcinoma "63." Th average weight of the tumours obtained in the treated mice when killed wa 3·37, 3·2, and 4·75 grammes respectively, as against 2·03, 2·38, and 2·2 respec tively in the control mice.

When the disintegration of the cells has been complete, not only does n immunity follow upon the inoculation of 0·10 to 0·50 c.c. of the disintegrate material, but in the majority of cases a distinct hypersensitiveness is induce in the animals so treated.

Varying the interval of time between the preliminary inoculation and th testing inoculation shows that this hypersensitiveness is not a phase ante cedent to the establishment of immunity. There is as little evidence o acquired resistance when the animals are tested at 30 days after th preliminary treatment as there is at 10 and 20 days.

The effect of inoculating varying doses of devitalised material has bee studied. When a scale of different doses ranging from 0·50 to 0·025 c.c. i inoculated, the weight of the tumours obtained from the testing inoculatio shows that 0·10 c.c. gave a greater average weight of tumour than larger o smaller doses. Although it would appear that the intermediate dose o 0·10 c.c. offers optimum conditions for hypersensitiveness, even so large a dos as 0·50 c.c. does not induce any increased resistance as compared with th normal animals of the same experiment. This is a relatively enormous dose and its inefficacy as an immunising agent shows that the absence of immunit after the absorption of dead material is not merely a question of too smal a dose.

Having established that the immunising power of tumours is abolished b mechanically injuring the cells (*i.e.* with the minimal chemical alteration i them), so that no growth ensues, the question next arises if the immunit obtained by normal tissues runs the same course. Fig. 1 shows that this i the case. In this experiment different batches of mice of about the sam age were treated, one set with an emulsion of fresh total mouse-embryo another with the same total mouse-embryo emulsion, ground in a mortar a a temperature of 0° C. for $1\frac{1}{2}$ hours; a third batch was treated with fresl

Experiment.	Preliminary inoculation. Material.	Preliminary inoculation. How treated.	Preliminary inoculation. Dose.	Preliminary inoculation. Date.	Preliminary inoculation. Remarks.	Interval between treatment and testing inoculation.	Testing inoculation. Tumour.	Testing inoculation. Dose.	Testing inoculation. Date.	Result. No. of tumours.	Result. No. of mice.	Result. Percentage.	Analysis of result when mice killed. Days after inoculation when killed.	Analysis. No. of tumours.	Analysis. No. of mice.	Analysis. Total weight of tumours.	Analysis. Average weight of tumours. *Pro* mouse.	Analysis. Average weight of tumours. *Pro* tumour.	Analysis. Average weight of mice.
1	63 carc.	Ground at the temp. of liquid air	0 ·10	6.1.09	No growth	Days. 15	63 carc. (Exp. 23B)	gr. 0 ·02	21.1.09	11	11	Per cent. 100	Days. 32	8	9	30 ·30	3 ·37	3 ·8	15 ·99
					Controls...	...	—	—	—	8	10	80	—	8	10	20 ·30	2 ·03	2 ·54	18 ·24
2	As above	—	—	—	No growth	17	63 carc. (Exp. 24A)	c.c. 0 ·025	23.1.09	10	10	100	34	9	9	28 ·85	3 ·21	3 ·21	16 ·4
					Controls...	...	—	—	—	9	10	90	—	8	9	21 ·40	2 ·38	2 ·68	17 ·43
3	As above	—	—	—	No growth	20	63 carc. (Exp. 23D)	c.c. 0 ·05	26.1.09	8	8	100	35	8	8	38 ·0	4 ·75	4 ·75	17 ·97
					Controls...	...	—	—	—	8	9	89	—	7	8	17 ·30	2 ·2	2 ·47	18 ·5

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mouse-embryo skin; a fourth with mouse-embryo skin ground at a temperature of about 0° as above. Fourteen days later all these mice, with controls corresponding, were inoculated with 0·01 c.c. of carcinoma "63." Fig. 1 shows the result of this experiment as observed on the 10th, 17th, 24th, and 31st day after the inoculation. In the control mice there are 17 tumours in 18 mice, *i.e.* 94 per cent. The mice treated with fresh embryo-emulsion

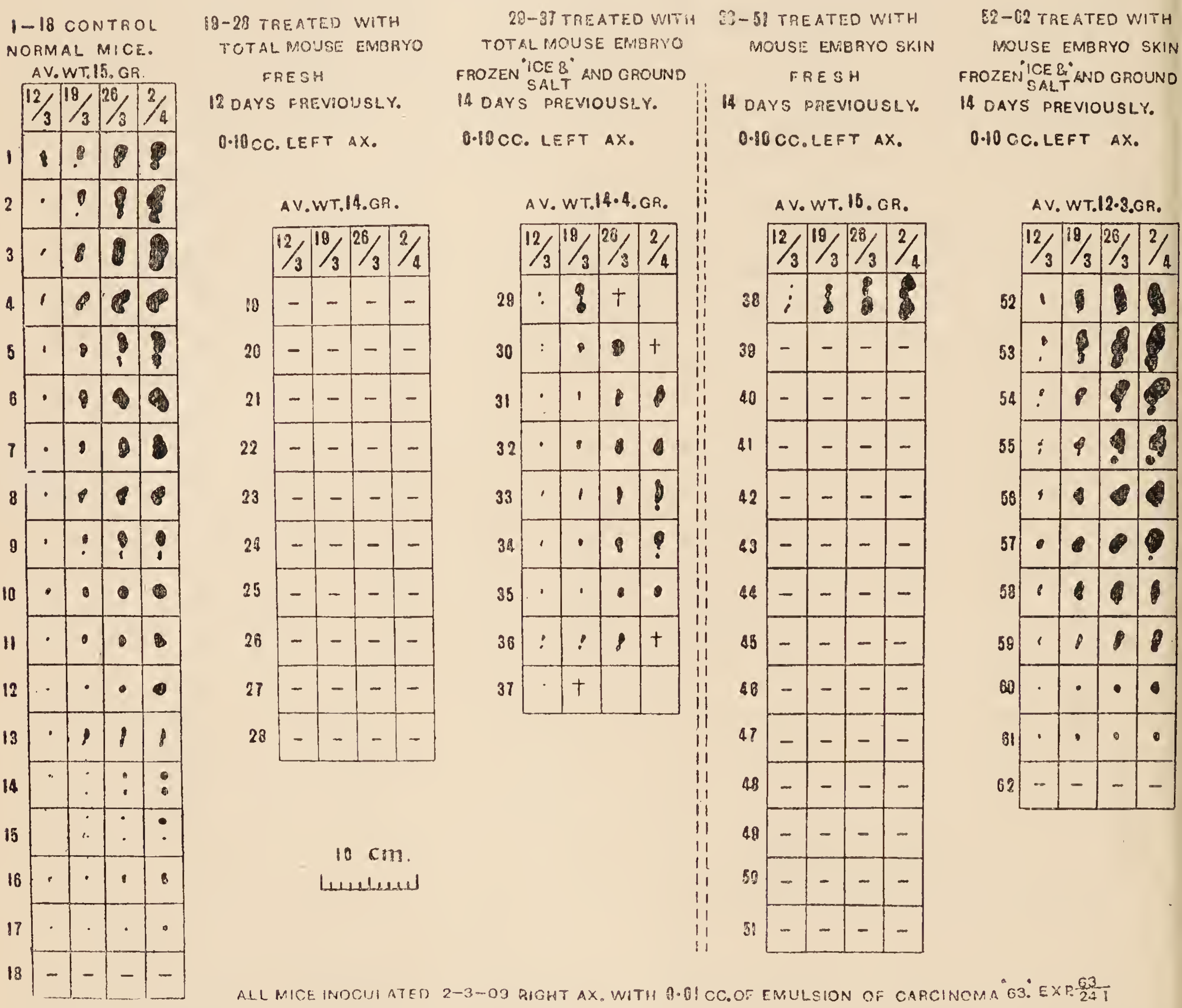

FIG. 1.—Experiment 63/24 I, showing the immunising effect of preliminary treatment of mice with normal tissues and the abolition of this effect after disintegration of the tissues by freezing and grinding. The numbers represent individual mice treated as stated at the top of each column. The silhouettes represent to scale the sizes of the tumours obtained in each animal at the dates stated, 10, 17, 24, and 31 days after inoculation. Negative results are represented by –.

are completely protected, whereas the mice of the third column, treated with ground embryo, developed tumours in each case, *i.e.* 100 per cent. The somewhat slower growth of the tumours in the third column, as compared with the controls, may be attributed to an infection, which killed several mice in the same cage, as shown on the chart. The mice treated with fresh skin gave one tumour in 14 mice (*i.e.* 7 per cent.), whereas the corresponding batch

treated with skin ground at a temperature of about 0° C. gave 10 tumours in 11 mice, *i.e.* 91 per cent., and these tumours grew more rapidly than the control tumours. This experiment demonstrates that an efficient disintegration of the cells of normal tissues robs them of all immunising properties. We have later repeated this experiment with other normal tissues, spleen, liver, and blood, with the same result.

Another method for obtaining the proteids of the cells has been tried: that of obtaining the press-fluid from tumours and normal tissues by Buchner's press after disintegration of the cells by grinding in a mortar with sand. The effect of a preliminary inoculation of such press-fluid on a subsequent inoculation of cancer was studied. Injection of 0·50 c.c. of tumour press-fluid, 11 days previous to testing inoculation, far from having any immunising effect, on the contrary hypersensitises the animals.

These experiments with ground material and press-fluid, both of cancerous and normal tissues, show that the power of inducing resistance is not bound up with the proteid of the cell as a chemical entity, but in some way or other depends upon properties of the living cells.

The investigations described above refer especially to mechanically disintegrated cells, but the results also apply to disintegration by autolysis, by heat, and to the influence of radium. In this latter case the microscopical appearance of the cells and their anatomical structure remains apparently unaltered. This shows that the loss of immunising power is not bound to any special form of disintegration, but is common to all means by which the life of the cell is destroyed.

The conclusions are :—

Complete disintegration of the tumour cells robs them entirely of their immunising properties.

There is no difference between tumour cells and normal cells in this respect.

The absence of immunising power does not seem to be a question of dose of introduced material, because relatively enormous doses of dead material (*e.g.* 1/26 of the weight of the animal) do not induce any resistance, whereas minimal doses of living cells (*e.g.* 1/1300 of the weight of the animal) have this effect.

The immunising property is not bound up with the protein of the cell, but depends on a different principle. Living cells are necessary to induce resistance to transplantation of cancer. It seems necessary that these cells must not only remain alive, but also even grow for a certain time; without the fulfilment of these conditions the reaction inducing active resistance is not set up.

The reaction which introduction of disintegrated cells calls forth is n only quantitatively different from that induced by living tissues, but als qualitatively different. Far from inducing any increased resistance, inocu lation of disintegrated cells only seems to manure the soil for a subsequen growth of tumours.

The failure to elicit the reactions of immunity to the transplantation cancer by devitalised tissues reveals an important difference from the immunit reactions obtained against bacteria and their products and foreign proteids i general, in which cases the immunising properties are independent of th vitality of the organisms or cells.

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